

## **IN THE SPECIFICATION**

Kindly enter the following paragraphs.

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~~Fig. 6.~~ Figs. 6A-B. Sequence alignment of the *rpoB* region amplified using a set of primers RPO5' and RPO3' from 35 different mycobacterial species. Sequences were aligned using multi-align program(6). Dashed lines represent nucleotide gaps.

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~~Fig. 7.~~ Figs. 7A-C. Examples of PCR-dot blot hybridization experiments. A total of 48 PCR products generated by using primers, RPO5, and RPO3', and DNAs from 48 mycobacterial species were blotted on the membrane, and an oligonucleotide probe which is specific to a certain mycobacterial species was hybridized at conditions described in the ~~Materials~~ Materials and Methods section. Blotted DNAs on the membrane were as following; 1: *M. tuberculosis*, 2: *M. scrofulaceum* 3: *M. szulgai*, 4: *M. gastri*, 5: *M. kansasii* type I, 6: *M. kansasii* type II, 7. *M. kansasii* type III, 8: *M. kansasii* type IV, 9: *M. kansasii* type V, 10: *M. terrae*, 11: *M. avium*, 12: *M. intracellulareae*, 13: *M. africanum*, 14: *M. celatum* type I, 15: *M. celatum* type II, 16: *M. haemophilum*, 17: *M. malmoense*, 18: *M. bovis*, 19: *M. chelonae*, 20: *M. abscessus*, 21: *M. ulcerans*, 22: *M. marinum*, 23: *M. genevane*, 24: *M. simiane*, 25: *M. flavescens*, 26: *M. fortuitum* type I, 27: *M. fortuitum* type II, 28: *M. peregrinum*, 29: *M. triviale*, 30: *M. phlei*, 31: *M. parafortuitum*, 32: *M. vaccae*, 33: *M. aurum*, 34: *M. neoaurum*, 35: *M. fallax*, 36: *M. xenopi*, 37: *M. aichiense*, 38: *M. mucogenicum*, 39: *M. nonchromogenicum*, 40: *M. senegalense*, 41: *M. smegmatis*, 42: *M. thermoresistable*, 43: *M. intermedium*, 44: *M. gordonae* type I, 45: *M. gordonae* type IL, 46: *M. gordonae* type III, 47: *M. gordonae* type IV, 48: *M. bovis*, BCG.

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Next, we sequenced PCR amplified region of the *rpoB* gene derived from 30 mycobacterial species that are known to have clinical importance. Subsequently, the sequences of the amplified regions were analyzed by using a software program (6). The result of the sequence analysis clearly showed that in the region of the *rpoB* we amplified, highly polymorphic regions exist, which are highly species-specific (~~Fig. 6~~) (Figs. 6A-B). This observation suggested to us that this highly polymorphic region of the *rpoB* can be very useful to design mycobacterial species-specific oligonucleotide probes, which can be used for developing a new PCR-dot blot hybridization technique for mycobacterial species identification. Subsequently, based on the sequence information, species-specific oligonucleotide was designed (Table 3), and each oligonucleotide was used as a probe in PCR-dot blot hybridization (~~Fig. 7~~). (Figs. 7A-C) In this experiment, a total of 48 mycobacterial species were blotted on the membrane, and each oligonucleotide was used as a probe to detect specific mycobacterial species. In brief, the results showed that each oligonucleotide probe was shown to be highly specific to each mycobacterial species targeted, indicating the usefulness of oligonucleotides for developing probe-based mycobacterial identification systems such as PCR-dot blot hybridization and PCR-reverse blot hybridization techniques.